PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of

Qiu-Ping QIN et al.

Serial Number: 10/580,329 Group Art Unit: 1641

Filed: May 24, 2006 Examiner: Grun, James Leslie

For: IMPROVED METHOD FOR DIAGNOSING ACUTE CORONARY SYNDROME

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

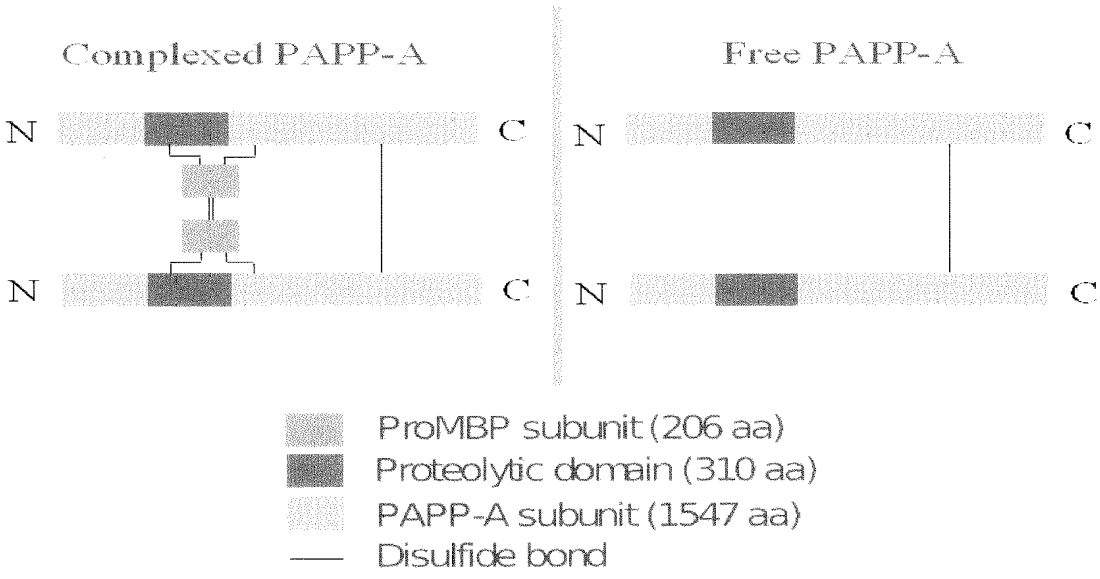
I, Kim Pettersson, PhD, hereby declare as follows:

1. I am a professor in Biotechnology at University of Turku, Department of Biotechnology, which is focused on research on *in vitro* diagnostic methods. During my 30 year career my research efforts have been devoted to the development of novel immunoassays applied to numerous human clinical areas. I have authored more than 200 peer-reviewed publications and, during the last fifteen years, have supervised about 20 doctoral thesis projects. Technological and clinical research for improved cardiac markers has been my primary interest for the last 12 years.

2. I am a co-inventor of the present invention, and have read and understand the claims of the above-captioned U.S. patent application ("this application"). The use of pregnancy-associated plasma protein ("PAPP-A") as a new cardiac marker is one of our most exciting research leads.

3. "Free PAPP-A", as defined in this application, is a form of PAPP-A which is not complexed with the proform of eosinophil major basic protein ("proMBP").

4. "Total PAPP-A", as defined in this application, is the sum of free PAPP-A and PAPP-A/proMBP complex. Fig. 1 below schematically illustrates free PAPP-A and the PAPP-A/proMBP complex:



5. In pregnancy serum, PAPP-A exists predominantly (>99%) in complex with proMBP, in which each PAPP-A subunit is connected to a proMBP subunit by two disulfide bonds [Overgaard et al., 278 J Biol Chem 21067(2003)]. Low concentrations of circulating PAPP-A are also found in healthy, non-pregnant populations, with the PAPP-A consisting mostly, if not completely, of the PAPP-A/proMBP complex [Wittfooth et al., 52 Clin Chem. 1794 (2006)].

6. In contrast, PAPP-A circulating in patients suffering from acute coronary syndrome (ACS) is not complexed with proMBP [Qin et al., 51 Clin Chem 75 (2005)].

7. Highly purified PAPP-A obtained from pregnancy serum can be used to immunize mice, rabbits or sheep to raise polyclonal and monoclonal antibodies. As discussed above, PAPP-A present in pregnancy serum is in the form of PAPP-A/proMBP complex. Accordingly, antibodies raised in this way do not react with epitopes exclusively present and accessible on the surface of free PAPP-A because these epitopes are blocked by proMBP. Instead, antibodies obtained in this manner are reactive with epitopes present and accessible on the surface of the PAPP-A/proMBP complex. This means that following three types of antibodies can, in principle, be obtained when using PAPP-A/proMB complex as an immunogen:

Type 1 antibodies, which specifically react with epitopes on the surface of the proMBP portion of the PAPP-A/proMBP complex;

Type 2 antibodies, which react with epitopes on the surface of the PAPP-A portion of the PAPP-A/proMBP complex; and

Type 3 antibodies, which react with epitopes (mixed or conformational) consisting in part of the PAPP-A surface and proMBP surface of the PAPP-A/proMBP complex.

Thus, a polyclonal antibody raised with PAPP-A/proMBP complex represents a mixture of Type 1, Type 2 and Type 3 antibodies. An

early commercial polyclonal antibody (A230) was shown to react with both PAPP-A/proMBP complex and proMBP in western blot analysis [Oxvig et al., 268 J Biol Chem. 12243 (1993), more specifically, Figure 1, panel D and paragraph 3 of the right column on page 12244], indicating this antibody preparation contains both Type 1 and Type 2 antibodies. On the other hand, a monoclonal antibody raised with PAPP-A/proMBP complex is specifically reactive with only one type of epitope present on the surface of the PAPP-A/proMBP complex.

8. The PAPP-A assay described in U.S. Patent No. 6,500,630 to Conover et al. is a sandwich biotin-tyramide amplified ELISA which uses a PAPP-A polyclonal capture antibody and a combination of PAPP-A monoclonal antibodies for detection (col. 22, lines 48-55). As explained in paragraph Nos. 9-11 below, none of the antibodies used in the Conover et al. assay can react exclusively with free PAPP-A.

9. Conover et al. does not describe the preparation of the antibodies used in its assay. Instead, Conover et al. cite Oxvig et al., 1201 Biochim.Biophys.Acta. 415 (1994), and Qin et al., 43

Clin.Chem. 2323 (1997) for a description of the polyclonal and monoclonal antibodies employed, respectively.

10. Conover et al. used the polyclonal antibody A230 (page 416, left column, paragraph 2.1), which is the same polyclonal antibody A230 cited on page 2325, right column, first full paragraph, of Qin et al. This polyclonal antibody, generated from rabbits with purified PAPP-A/proMBP complex obtained from pregnancy serum, is commercially available from DakoCytomation Denmark A/S. A230 antibody reacts with both the PAPP-A subunit and the proMBP subunit of PAPP-A/proMBP complex. See the antibody specification in the attached DakoCytomation sheet.

11. The PAPP-A monoclonal antibody combination used by Conover et al. consists of monoclonal antibodies Hyb234-2, Hyb234-3, Hyb234-4, Hyb234-5 and Hyb234-6. See Qin et al., 43 Clin.Chem. 2323 (1997) at page 2324, right column, 2nd paragraph. These monoclonal antibodies were raised by immunizing Balb/C mice with PAPP-A/proMBP complex isolated from pregnancy serum and were all found to react with the PAPP-A subunit of PAPP-A/proMBP complex, and not the proMBP part. See Qin, "Maternal serum screening for Down

syndrome in the first trimester with special emphasis on pregnancy associated plasma protein A," PhD thesis, (1998), at Table 2 and Figure 1 of article II.

12. As explained above, the PAPP-A assay described in Conover et al. cannot exclusively detect free PAPP-A, as this is simply prohibited due to the specificities of the polyclonal and monoclonal antibodies used. Instead, as illustrated in Figure 2 below, the Conover et al. assay in fact detects total PAPP-A, which includes both free PAPP-A and also PAPP-A/proMBP complex.

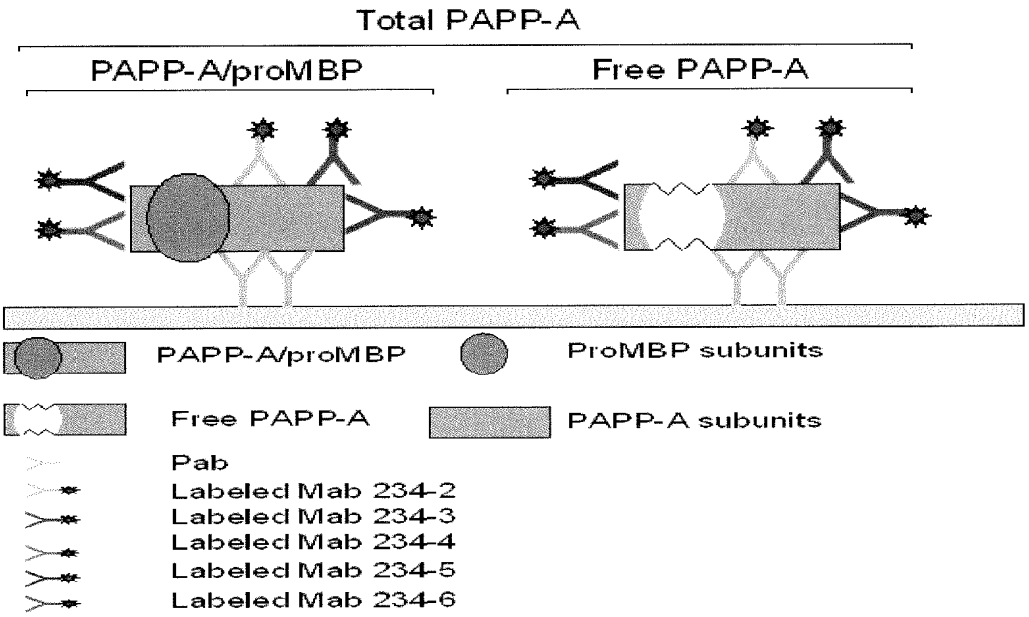


Figure 2. PAPP-A forms detected by the PAPP-A assay described in US 6,500,630.

13. In summary, the PAPP-A assay described in Conover et al. detects both free PAPP-A and PAPP-A/proMBP complex because the antibodies involved in the assay react with the epitopes simultaneously present on the surface of PAPP-A/proMBP complex as well as on the surface of free PAPP-A. The assay is unable to distinguish the PAPP-A/proMBP complex from free PAPP-A.

14. The inability to distinguish between free PAPP-A and the PaPP-A/proMBP complex is a serious clinical disadvantage of the assay disclosed in Conover et al. This is because ACS results in an increase in free PAPP-A. Therefore, it is natural that the clinical value (sensitivity, specificity, extent of useful time-window) explored in ACS patients with assays such as the one described in Conover et al. is likely to be seriously compromised.

15. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Signed this 16th day of April, 2009.

Kim Pettersson, PhD

Attachment:
DakoCytomation Sheet on Polyclonal Rabbit Anti-Human Pregnancy-Associated Plasma Protein A Code No. A0230